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HELMINTHOSPORIUM TURCICUM PASS.

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ON A METHOD OF SUBMERGED CULTIVATION OF *PIRICULARIA ORYZAE* BR. ET CAV. AND
HELMINTHOSPORIUM TURCICUM PASS.

[Following is the translation of an article by I.N. Miusov and Yu. V. Peruanskiy, published in the Russian-language periodical Vestnik Selskokhozyaystvennoy Nauki, (Alma-Ata) (Herald of Agricultural Science) Vol 8, No 12, 1965, pages 20-24. Translation performed by Sp/7 C. T. Ostertag, Jr.]

In the practice of plant breeding it is becoming more necessary to test the resulting types, hybrids and varieties of agricultural crops for resistance to specific diseases caused by phytopathogenic fungi. For this purpose the breeder should have a sufficient amount of standard infectious material.

At the present time the majority of facultative plant parasites are cultivated in small quantities (test tubes, small flasks) on solid nutrient media. However, such infectious material may introduce an element of chance when the breeder is carrying out extensive tests (frequent sub-cultures, nonuniformity of material obtained from different test tubes, flasks, etc.).

Nevertheless at the present time the method of submerged cultivation of microorganisms with forced aeration is being used on a large scale for the purpose of obtaining antibiotics, enzymes, amino acids, vitamins, etc.

A number of investigators (1-9) have demonstrated the successful cultivation of certain phytopathogenic fungi in liquid media, though in small volumes.

For obtaining a sufficient quantity of the infectious source it is necessary that the cultivation of phytopathogenic fungi be carried out in large vessels. This creates the necessity for the application of forced aeration.

In this report we will present the results of experiments on the cultivation of two facultative plant parasites in liquid media with uninterrupted aeration. These are *Piricularia oryzae*, which causes rice blast, and *Helminthosporium turcicum*, which infects corn.

Both fungi were isolated from diseased plants and obtained in a pure culture (by applying the methods generally accepted in mycology and phytopathology). For the purpose of turning out the initial material for the inoculation of liquid nutrient media *P. oryzae* and *H. turcicum* were propagated on nutrient media treated with agar. The main set-up for cultivating the fungi under study is shown in figure 1.

P. oryzae was cultivated in nutrient medium prepared from the digest of carrot root crops (1 kg of carrots per 4 liters of water). The pH of such a medium fluctuated from 5.9 to 7.0. The inoculum of fungus was added on the basis of 8% to the volume of medium. The temperature was maintained between 26 and 28°C. Aeration was carried out at the rate of 1 liter/minute of air per 1 liter of medium. Cultivation lasted for 8--9 days and by the end of this period we obtained up to 10 grams of air-dried mycelium of P. oryzae from one liter of nutrient medium. In 4--5 days one gram of such mycelium formed up to 60 million spores.

H. turcicum was incubated in liquid nutrient medium of the following composition: Corn extract - 5 grams, saccharose - 10 grams, vegetable oil - one gram, chalk - 0.5 grams, wafer - 1,000 grams, pH of the medium - 6.2. The inoculum of fungus was added on the basis of 4% to the volume of medium. The temperature was maintained between 22 and 26°C. Aeration was carried out at the rate of 0.8--1 liter/minute of air per 1 liter of medium. Cultivation lasted for 5--6 days and by the end of this period we obtained up to 7 grams of air-dried mycelium of H. turcicum from one liter of nutrient medium. In 4--5 days one gram of such mycelium formed up to 40 million spores.

The cultivation of mycelium in liquid nutrient medium with a continuous forced aeration and the subsequent obtaining of spore material from the mycelium which were isolated from the cultural liquid is not altogether a common phenomenon for facultative plant parasites. As is known, under natural conditions such fungi are nourished on a living organism and form spores which are capable of infecting new plants.

In connection with the fact that the spores of these two fungi were obtained under artificial conditions, we had the mission of clearing up to what extent such spores preserve their biological properties, primarily virulence. For checking the virulence of the resulting spore material, rice and corn plants were cultivated under hothouse conditions and in the open ground.

The virulence of *Piricularia* was checked by the generally accepted phytopathological method on a moderately susceptible rice variety - the Dungan-Shala. It was established by observations that the rice plants are infected by a virulent source of fungus cultured by the submerged method. In addition to the spores, the rice plant may be infected by mycelia when they are deposited on the surface of leaves together with cultural liquid.

Analogous experiments were carried out with *Helminthosporium* fungi on corn plants of the Odesskaya 10 variety. For the infection of the plants it is possible to use the mycelia from a submerged culture, spores obtained from such mycelia, and a mixture of both components (table 1).

It is apparent from table 1 that in the capacity of an infectious source it is possible to use pure *Helminthosporium* spores and the spores in a mixture with mycelia. Pure mycelium may also cause the infection of corn plants. It was established by additional tests that the mycelial hyphae, incubated

by the submerged method and having a length of from 0.2 up to 3.5 microns, are capable of germinating and implanting in the tissue of a leave.

Thus a check on plant-hosts showed that following the submerged cultivation of mycelium such material did not lessen in its initial virulence. In table 2 we present the results of checking the virulence of *Helminthosporium* spores obtained from an agar and submerged culture of the fungus.

The results of the tests showed that the quality of spores from a submerged culture is no lower than that of spores obtained from an agar culture. Liquid nutrient medium, when the necessary components are present in it and the culture has a sufficient supply of oxygen, is conducive to preserving the virulence of spore material. Besides this, spores formed on mycelium obtained in a liquid nutrient medium are morphologically equal in a comparison with spores which are characteristic for the mycelium from solid nutrient medium (figures 2 and 3).

Conclusions

1. A method is proposed for the cultivation of certain facultative parasites in a liquid medium with forced aeration. It may be used for turning out a sufficient amount of standard infectious material, which in turn could be used by breeders for appraising the resistance of plant types to specific fungal diseases.
2. Spore material obtained from mycelium of a submerged culture is homogeneous in its morphological features and does not lose its virulence.

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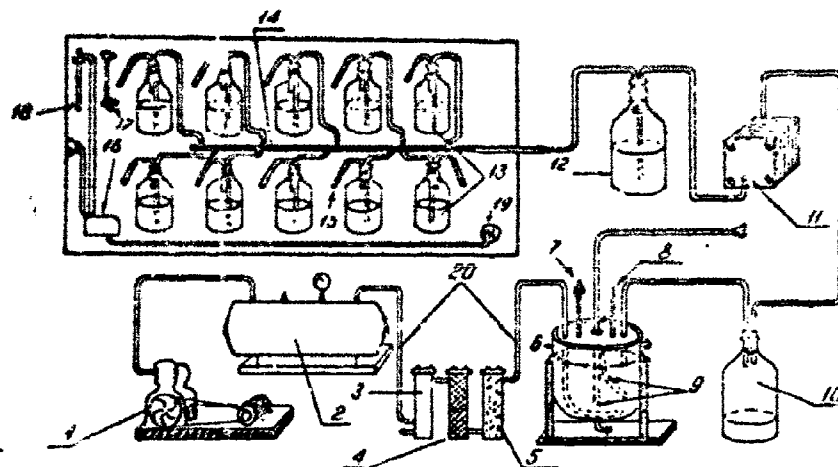


Figure 1. Arrangement of devices and units for the submerged cultivation of Piricularia oryzae and Helminthosporium turcicum.

Legend: 1 - garage type compressor; 2 - receiver; 3 - settling tank for collecting condensate; 4 - carbon filter; 5 - cotton filter; 6 - device for sterilization and heating air in winter; 7 - contact thermometer; 8 - control thermometer; 9 - heating elements; 10 - settling tank (collection of condensate); 11 - Seitz filter; 12 - air humidifier; 13 - cultivation vessels (5--20 liter capacity); 14 - air distributor; 15 - cotton filter; 16 - electronic relay; 17 - control thermometer; 18 - contact thermometer; 19 - heater; 20 - tubing.

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Fig. 2. Spores of *H. turcicum*, obtained from mycelium cultivated by the submerged method.

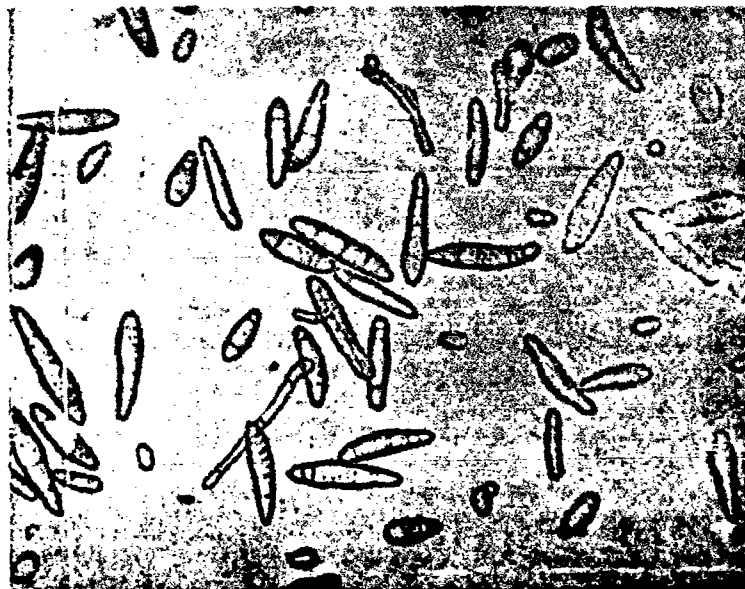


Fig. 3. Spores of *H. turcicum*, obtained from mycelium cultivated on agar solid medium.

Table 1

Infection of corn plants by Helminthosporium depending on the infectious source used

Type of infectious source	Number of infected plants (in %)
Spores	100.0
Mixture of spores and mycelium	100.0
Mycelium	50.0

Table 2

Comparison of the capability of Helminthosporium spores, obtained from agar and liquid cultures, to infect corn plants

Method of cultivating fungus	Number of infected plants (in %)
Submerged culture . . .	100.0
Agar culture	94.0